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RESEARCH PAPER

Development of gluten-free brown rice/soy protein/corn starch-based pasta enriched with Microalgae (*Haematococcus pluvialis*) flour produced via a cold extruder

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Abstract

The increasing trend of healthy awareness demands nutrient-rich food consumption, for instance, processed with no gluten. This research focused on developing gluten-free pasta utilizing a composite blend of brown rice, soy protein isolate, and corn starch, enriched with microalgae *Haematococcus pluvialis* (HP) flour. A 3×3 factorial design was used based on Feed Moisture Content (FMC) (36%, 40%, 44%) and HP supplementation levels (0.5%, 1.0%, 1.5%), with wheat protein as a control produced using cold extrusion techniques. The enriched pasta was characterized based on physicochemical properties, cooking quality, microstructure, antioxidant activity, and sensory attributes. The result presented that HP supplementation depicted yellow tone color and showed a significant increase in protein content (32.78–33.35%), fiber content (3.21–3.62%), and reduced carbohydrate content compared with the control group. The textural properties of uncooked pasta exhibited increased hardness with the addition of HP, indicating good material synergistic effects to inhibit physical cracks during storage. The DPPH radical scavenging activity increased significantly with the increase of HP by 64.1% (raw samples) and 40.7% (cooked samples). In addition, sensory evaluation revealed the highest overall acceptability at increment HP levels (0.5–1.0%) and a FMC of 40%. The findings indicated that HP could improve gluten-free pasta's nutritional and functional characteristics while ensuring consumer acceptability when optimized for concentration and moisture levels.

Keywords

Agriculture innovation, antioxidant activities, astaxanthin, extrusion, food production, *Haematococcus pluvialis*, gluten-free pasta

Introduction

Pasta is a globally recognized traditional food product for its convenience, affordability, extended shelf life, and nutritional benefits, especially its complex carbohydrate composition and potential for fortification with protein, fiber, vitamins, and minerals. (Chillo et al. 2008; Giacco et al. 2015; Kamble et al. 2019). Pasta is traditional-

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ly produced by combining durum wheat semolina with water. Durum wheat, a gluten-containing cereal from the *Triticum durum* family, is remarkably esteemed for its pasta-making qualities, attributed to the viscoelastic properties conferred by gluten, a protein complex mainly consisting of gliadin and glutenin (Arendt and Dal Bello 2009; Murray et al. 2017).

Gluten is essential for influencing the structure, texture, elasticity, and cooking quality of pasta (Zou et al. 2016; Caratini and Rosentrater 2019). Nonetheless, despite these advantageous characteristics, gluten may adversely affect individuals with gluten-related disorders. Celiac disease (CD) is an immune-mediated inflammatory disorder triggered by gluten intake, leading to damage of the intestinal villi and presenting symptoms such as diarrhea, bloating, and nutrient malabsorption. CD impacts around 0.3–1% of the worldwide population, with variations observed based on age, sex, and geographic location (Stojceska et al. 2010; King et al. 2020). Accordingly, wheat pasta typically possesses a high glycaemic index, leading to swift increases in blood sugar levels, which may be unsuitable for individuals with diabetes or those seeking to regulate insulin levels. Refined wheat pasta is often low in dietary fiber and essential micronutrients unless it is fortified (Husby et al. 2012; Sosa et al. 2019). Furthermore, an increasing population experiences non-celiac gluten sensitivity (NCGS), a condition that double-blind, placebo-controlled studies have recently substantiated (Volta et al. 2013). For these populations, even minimal gluten exposure can provoke adverse reactions, necessitating a strict lifelong gluten-free diet as the sole effective treatment.

The rising incidence of gluten intolerance and celiac disease has increased demand for high-quality gluten-free alternatives to conventional wheat-based pasta (Aljada et al. 2021). However, producing gluten-free pasta poses considerable challenges, while the lack of gluten affects the structural integrity of pasta, frequently leading to suboptimal texture, reduced elasticity, and inadequate cooking characteristics. Gluten is essential for forming the viscoelastic network of dough and preserving cooking integrity. Therefore, the removal of gluten material requires alternative ingredients and novel processing techniques to ensure satisfactory product quality (Ramos et al. 2023). The increasing demand has prompted significant research into the creation of gluten-free pasta utilizing diverse raw materials and technologies designed to replicate the functional properties of gluten. Research frequently focuses on plant-based flours, hydrocolloids, and protein fortifiers to enhance the nutritional, sensory, and structural qualities of gluten-free pasta (Khatri et al. 2023; Pinel et al. 2024; Delgado-Pando et al. 2025).

Brown rice (BR) is a whole grain that is nutrient-dense and contains high levels of antioxidants, fiber, and bioactive compounds, including γ-oryzanol, γ-aminobutyric acid (GABA), and phenolics, which provide anti-diabetic and cardioprotective effects (Rafiq et al. 2017; Bernas et al. 2025). Nonetheless, the low protein content, absence of gluten, and firm texture restrict its application in processed products such as pasta, frequently leading to diminished elasticity, extended cooking durations, and inferior sensory quality (Udachan et al. 2022). To mitigate these deficiencies, soy protein isolate (SPI) is commonly incorporated due to its superior nutritional value and functional characteristics such as emulsification, gelation, and water/oil absorption which enhance protein content, texture, and decrease cooking time and cooking loss, while also addressing the dietary requirements of specific populations, including the elderly and diabetics, by promoting better glycaemic control (Ojukwu et al. 2022; Liang et al. 2023; Qiao et al. 2023). Additionally, corn starch (CS) enhances pasta quality by improving softness, stretchability, and decreasing cooking time and cooking loss (Liu et al. 2021; Zhu et al. 2024; Xue et al. 2025). The elevated amylose content facilitates the formation of resistant starch and engages with non-starch components through hydrogen bonding, thereby enhancing nutritional value, texture, and structural integrity in gluten-free formulations (Saleh et al. 2017). Integrating BR, SPI, and CS hypothetically could create a complementary matrix that enhances gluten-free pasta's technological, nutritional, and sensory attributes.

Moreover, microalgae are receiving heightened interest in the food industry as sustainable, nutrient-rich ingredients, mainly because of their elevated protein levels and bioactive compounds (Xia et al. 2022). HP is a nutrient-dense freshwater microalgae recognized for its potential as a functional ingredient, primarily due to its significant astaxanthin content, constituting approximately 69.7% of its carotenoids. Additionally, it contains 27% lutein and 3% β-carotene, contributing to its antioxidant properties and mild seaweed aroma (Vidhyavathi et al. 2007; Liu et al. 2023). HP comprises 48% protein, 27% carbohydrate, and 15% lipid, providing nutritional and color-enhancing advantages in food applications. Traditionally utilized in aquaculture and poultry for pigmentation, direct incorporation into foods offers a cost-effective method to harness its antioxidant and health-promoting properties (Fu et al. 2021). In line with post-COVID shifts in food microbiology and bioeconomics, the incorporation of HP in pasta should be interpreted not only as a technological innovation but as a bioeconomic strategy. Bibliometric evidence shows a growing, though still fragmented, convergence between bioeconomy and food microbiology aimed at eco-intensification and resilient, health-oriented food systems (Zuniga-Gonzalez 2025). Microalgae have emerged as key bioeconomic resources: they provide renewable, nutrient-dense biomass with valuable proteins, pigments such as astaxanthin, and other bioactives, while requiring limited land and water and fitting into circular, low-carbon production models. Positioning HP within a widely consumed staple like pasta therefore, responds to global calls for 'future foods' that can simultaneously enhance nutritional quality, reduce environmental burdens, and strengthen the robustness of food supply chains in the post-pandemic context. Research indicates that HP could enhance antioxidant capacity and glycaemic response in products such as cookies. While in soy protein-based high-moisture meat analogs (HMMA), the addition of HP (0.25–1.25%) improved hardness, chewiness, and re-

silience. Sensory analysis indicated that 1% HP produced a beef-like color, whereas 0.25% exhibited characteristics similar to pork (Mofasser Hossain et al. 2017; Huang et al. 2024). HP demonstrated consistent color stability when subjected to light exposure, freeze/thaw cycles, cooking, and consumer acceptance, indicating its viability as a potential natural colorant and functional additive in plant-based and gluten-free food products.

Extrusion technology is extensively utilized in food manufacturing to generate uniform, shelf-stable products with a favorable texture. Mishra, Mishra and Srinivasa Rao, (2012) categorized extrusion processes into two primary types: cold extrusion and hot extrusion, determined by the level of thermal energy utilized in the process. Cold extrusion is a low-temperature forming process for conventional pasta. The processing temperature generally stays below 70 °C, considerably lower than that observed in hot extrusion methods. Cold extrusion is suitable for applications that require preserving the native structure or functionality of heat-sensitive ingredients. This process facilitates the formation of products with specific shapes and textures, including pasta, noodles, and restructured rice (Patria et al. 2021; Itusaca-Maldonado et al. 2024; Patria et al. 2024; Suo et al. 2024; Zeng et al. 2025). Due to incomplete gelatinization or denaturation, further post-processing steps, such as drying or cooking, may be necessary to attain the final desired texture or ensure safety for consumption.

Therefore, this research focuses on developing gluten-free pasta using a composite flour blend comprising brown rice, soy protein, and corn starch, supplemented with varying concentrations of HP, facilitated by a cold (pasta) extruder. The products were evaluated based on the physicochemical properties, cooking quality, microstructure, antioxidant activity, and sensory attributes. This study determined the viability of HP as a functional component in gluten-free pasta and its implications for health-focused food innovation.

Materials and methods

Materials

The materials used to produce gluten-free pasta consist of brown rice (BR) obtained from Sun Da Rice International Co., Ltd in Kaohsiung, Taiwan. Soy protein isolate (SPI) was obtained from Archer Daniels Midland (ADM) Company, USA. Isolated Wheat Protein (IWP) was sourced from Manildra Group, USA, and Corn Starch (CS) was obtained from Ingrain SA (Pty) Ltd., South Africa. HP flour is sourced from Changsa Herbway Biotech Co., Ltd, China. The employed chemicals included boric acid (Union Chemical Works, Ltd., Taiwan), hydrochloric acid (Sigma-Aldrich, Germany), sulfuric acid (Sigma-Aldrich, Germany), copper (II) sulfate pentahydrate (Showa Chemical, Japan), potassium sulfate (Showa Chemical, Japan), methyl red and bromocresol green (Koch-light Laboratories, UK), ethyl ether (Echo Chemicals, Taiwan), reverse osmosis water, and hexane.

Experimental design

The research examined the impact of two processing variables: FMC at three levels (36%, 40%, and 44%) and the concentration of HP flour supplementation at 0.5%, 1.0%, and 1.5% (based on a total composite flour, wet basis). Ten experimental trials were conducted, which included one control sample that utilized wheat protein in the production process (Table 1). Specific treatments were performed in triplicate, whereas the texture profile was evaluated with ten replications. A variety of physicochemical characteristics were assessed, including proximate analysis, color analysis, texture profile, water absorption index (WAI), water solubility index (WSI), optimum cooking time (OCT), water absorption ratio (WAR), cooking loss (CL), microstructure, DPPH free radical scavenging, and sensory evaluation.

Preparation, mixtures, and extrusion process of gluten-free pasta

Initially, brown rice undergoes processing with a grinder (model RT-CV50S, Rong Tsong Precision Technology Co., Ltd., Taiwan) and is subsequently sieved utilising an 80-mesh sieve. According to the established research design, brown rice flour is subsequently combined with additional components, including SPI, CS, and HP (Table 1). The water content is assessed upon combining all ingredients to ascertain the FMC. Subsequently, to attain pre-gelatinization of the flour, add boiling water based on the moisture content outlined in equation (1). After mixing the materials is uniformly stirred and readied for the extruder machine.

$$\mathbf{FMC} = \left(\frac{(\mathbf{M_0} \times \mathbf{M_1}) + \mathbf{M_2}}{\mathbf{M_0} + \mathbf{M_2}} \right) \quad (1)$$

In this context, FMC represent Feed Moisture Content, M_0 denotes the total weight of mixed flour utilized, M_1 indicates the moisture content of the mixed flour, and M_2 refers to the quantity of water required to attain the specified FMC.

The pasta was produced using a modified method based on the approaches outlined by Khatri et al. (2023) and Patria et al. (2021). Following the mixing of all flours with boiling water and the adjustment of the FMC by the design specifications, the mixture was introduced into a single-screw

Table 1. Experimental design.

No	Sample	Blend Composition (%)					FMC (%)
		IWP	BR	SPI	CS	HP	
1	Control	15	60	15	10	-	48
2	HP 0.5 FM 36	-	60	30	10	0.5	
3	HP 1 FM 36	-	60	30	10	1	36
4	HP 1.5 FM 36	-	60	30	10	1.5	
5	HP 0.5 FM 40	-	60	30	10	0.5	
6	HP 1 FM 40	-	60	30	10	1	40
7	HP 1.5 FM 40	-	60	30	10	1.5	
8	HP 0.5 FM 44	-	60	30	10	0.5	
9	HP 1 FM 44	-	60	30	10	1	44
10	HP 1.5 FM 44	-	60	30	10	1.5	

IWP: Isolated Wheat Protein; BR: Brown Rice; SPI: Soy Protein Isolate; CS: Corn Starch; HP: *Haematococcus Pluvialis*; FMC: Feed Moisture Content.

cold (pasta) extrusion machine (La Monferrina Model P6, Rome, Italy) featuring an open die configured for pasta, designated with die code 420. The length of the screw and the diameter of the machine were 36 cm and 5 cm, respectively. The cold extrusion process employed in this study does not allow independent control of barrel temperature or screw speed; however, this design ensures stable. Following the extruder process, the samples were cut to approximately 4 cm using the machine's electric cutter after emerging from the die. For a more detailed process, refer to Fig. 1. Following collection, the fresh pasta was transferred to an air dryer for the drying process at a temperature of 45 °C for 2 h. Following the drying process, the dried pasta was collected (Fig. 4) and then packed in a Ziplock bag made of low-density polyethylene (LDPE) and stored at room temperature (25–28 °C). A laboratory grinder (model RT-04, Mill Powder Tech Co., Ltd., Taiwan) was employed to grind 100 g of dried pasta, randomly selected from all extruded samples, into powder. The resulting powder was then sieved using an 80-mesh sieve for physicochemical structure analysis.

Proximate analysis

The determination of crude protein content (AOAC method 978.04), crude fat content (AOAC method 930.09), moisture content (AOAC method 925.09), and ash content (AOAC method 930.05) was conducted following the AOAC (2005) methodology. The total carbohydrate content was identified as the distinguishing factor between these results.

Colour measurement

The colour properties of pasta were assessed using the procedure outlined by Patria et al. (2023) with a colorimeter (Hunter Lab, Colour Quest XE, USA). Whereas, a^* represents red-green coordinates, with negative values indicating green and positive values indicating red. Conversely, b^* denotes blue-yellow coordinates, where negative values signify blue and positive values signify yellow. L^* is a measure of brightness, ranging from 0 to 100.

Texture profile analysis

Texture analysis was conducted following the modified method reported by Jia et al. (2023). The pasta was divided into two portions: the first portion consisted of dried pasta (uncooked) to assess hardness, while the second portion consisted of wet pasta (cooked) to evaluate cohesiveness, springiness, chewiness, and gumminess. Each pasta sample was cooked for the optimal duration, afterward dried, and rested for 5 min at room temperature before being measured using a texture profile analyzer (5564, Instron Co., Massachusetts, USA). Ten strands of uncooked and cooked pasta measuring 3 cm in length were arranged parallel on a metal plate with an approximate spacing of 3 mm. A cylindrical probe with a diameter of 36 mm was employed to compress the paste twice, simulating chewing behaviour. The compression level reached 75% at a 2 mm/min rate.

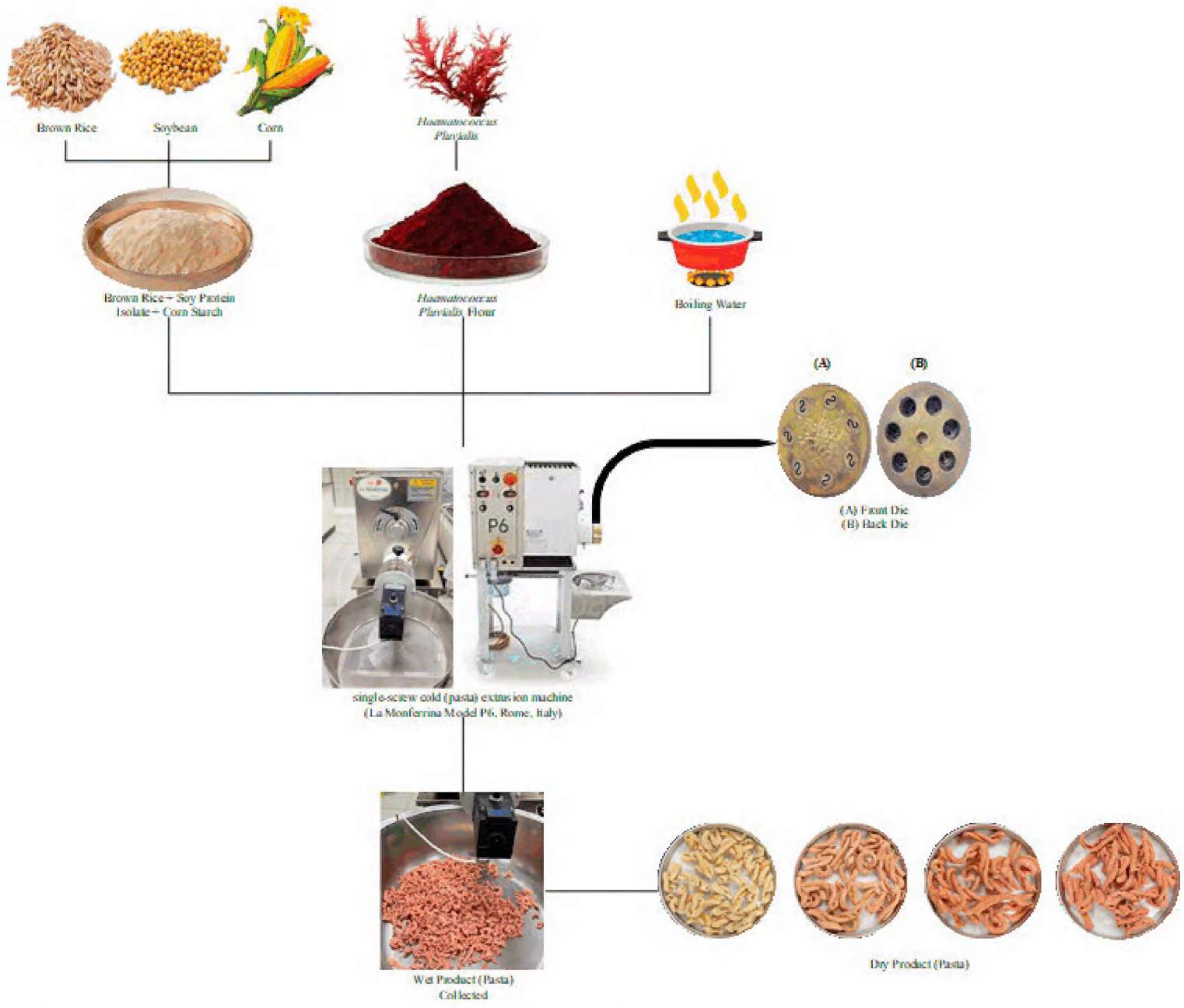

Figure 1. A schematic representation of the gluten-free pasta production proce.

Water Absorption Index (WAI) and Water-Soluble Index (WSI)

The water absorption index (WAI) and the water-soluble index (WSI) were determined using the methods described by Patria et al. (2021). The sample flour (2 g) was mixed with 20 mL of distilled water in 50 mL centrifuge tubes, agitated for 10 min, and then centrifuged for 20 min at 3000 rpm. The supernatant was then transferred to an aluminium dish and dried for 24 h at 105 °C in the oven. The weights of the desiccated supernatant and gel were measured. Equations (1) and (2) were utilised to calculate WAI and WSI, respectively:

$$\text{WAI}\left(\text{g}'\text{g}\right) = \frac{\text{weight of sample gel(g)}}{\text{weight of sample flour(g)}} \quad (2)$$

$$\text{WSI}(\%) = \frac{\text{weight of dried supematant(g)}}{\text{weight of sample flour(g)}} \times 10 \quad (3)$$

Optimum Cooking Time (OCT), Water Absorption Ratio (WAR), and Cooking Loss (CL)

The estimated optimal cooking time, WAR, and CL of pasta were determined using a modified method outlined by Xiong et al. (2025). The optimum cooking time was defined as the period required for the white core at the center of the pasta to disappear when compressed between two glass plates, with assessments conducted every 30 s. For the measurements of WAR and CL, 2 g of pasta were cooked in 20 mL of reverse osmosis (RO) water for the established optimal cooking duration. Following the cooking process, the pasta was drained, and the supernatants were gathered and placed in an aluminium dish, subsequently dried at 105 °C for 24 h. The weight of the cooked pasta was measured to calculate the WAR, while the dried supernatants were utilised to assess the CL. Equations 4 and 5 below present the formulas for calculating WAR and CL:

$$\text{WAR}(\%) = \frac{\text{weight of cooked pasta(g)-weight of uncooked pasta(g)}}{\text{weight of uncooked pasta(g)}} \times 10 \quad (4)$$

$$\text{CL}(\%) = \frac{\text{weight of dried supernatant(g)}}{\text{weight of uncooked pasta(g)}} \times 100 \quad (5)$$

Microstructure

The microstructure of pasta was determined using the modified method described by Patria et al. (2024). The microstructures of pasta were examined with a scanning electron microscope (SEM) (S-3000 N, Hitachi High Technologies Co., Japan) at magnifications of 500×. Before the SEM analysis, the samples were subjected to cooking for the predetermined optimal duration and subsequently dried for 48 h using a freeze dryer (FD-1200, EYELA Co., Ltd., China) to reduce moisture content.

DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging of the samples was assessed using the method described by Bernas et al. (2025). A 0.5 g flour was extracted with 25 mL of methanol for 1 h and centrifuged at 7,000 rpm for 15 min. Five hundred microlitres (500 µL) of the upper layer was collected and combined with 500 µL of 0.2 mM DPPH solution. The solution should be maintained in darkness at room temperature for 30 minutes to prevent exposure to light, followed by centrifugation at 10,000 rpm for 10 min. 190 µl of supernatant and blank were aliquoted into a 96-well plate. An ultraviolet (UV)-visible spectrophotometer (Epoch; BioTek Instruments) measured absorbance at 517 nm following a 30-min interval. The DPPH free radical scavenging capacity was determined using the following equation (6):

$$\text{DPPH Free Radical Scavenging}(\%) = 1 - \frac{\text{absorbance sample}}{\text{absorbance blank}} \times 100\% \quad (6)$$

Sensory evaluation

Thirty untrained students aged approximately 18 to 25 years old from Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia, performed sensory evaluations of gluten-free pasta. Participants without a history of gluten allergy were provided with five pieces of pasta for each treatment, including the control, prepared according to the designated optimal cooking time. The hedonic test utilized a scale with the following values: 1 = very dislike, 2 = slightly dislike, 3 = like, 4 = slightly like, and 5 = very like. Sensory evaluation encompasses factors such as taste, color, aroma, texture, and overall acceptability. The sensory analysis was conducted as a preliminary consumer acceptability test using an untrained panel to support early-stage formulation development rather than to provide population-level consumer preference data.

Statistical analysis

Quantitative data were analysed through analysis of variance (ANOVA) and subsequently assessed using Duncan's Multiple Range Test (DMRT) at a 95% confidence inter-

val ($p < 0.05$). Statistical analysis utilised SPSS (Statistical Package for the Social Sciences Version 22.0, SPSS Inc., Chicago, IL, USA). Pearson correlations among responses were assessed utilising OriginLab Pro version 2025 (Northampton, Massachusetts, USA).

Results and discussion

Proximate composition

The impact of HP concentration and FMC on the chemical composition of gluten-free pasta is described in Table 2. The supplementation with HP led to a significant reduction in the carbohydrate content of gluten-free pasta ($p < 0.05$), decreasing from 64.24% in the control group to a range of 63.41–63.89% in the HP treatments. In contrast, FMC (36–44%) demonstrate no significant effect ($p > 0.05$). This indicates that the observed reduction in carbohydrates is attributable to formulation changes, specifically the partial replacement of starch-rich ingredients with HP, rather than to variations in extrusion moisture conditions. Similarly, Kumoro et al. (2016) suggested that algae-fortified noodles presented increasing macronutrient and lower carbohydrate content.

Table 2. Chemical composition of gluten free pasta (dry basis)[1,2].

Sample	Carbohydrate (%)	Protein (%)	Ash (%)	Fat (%)	Crude Fiber (%)
Control	64.24 ± 0.2^{a}	32.78 ± 0.3^{d}	0.98 ± 0.4^{a}	2.03 ± 0.3^{b}	3.21 ± 0.2^{d}
HP 0.5 FM 36	63.70 ± 0.3^{b}	33.11 ± 0.2^{c}	0.89 ± 0.2^{b}	2.31 ± 0.2^{a}	3.45 ± 0.4^{c}
HP 1 FM 36	63.55 ± 0.4^{c}	33.24 ± 0.3^{b}	0.89 ± 0.6^{b}	2.32 ± 0.1^{a}	3.52 ± 0.5^{b}
HP 1.5 FM 36	63.44 ± 0.1^{d}	33.33 ± 0.4^{a}	0.91 ± 0.4^{b}	2.32 ± 0.5^{a}	3.61 ± 0.4^{a}
HP 0.5 FM 40	63.74 ± 0.6^{b}	33.09 ± 0.1^{c}	0.88 ± 0.2^{b}	2.30 ± 0.5^{a}	3.44 ± 0.3^{c}
HP 1 FM 40	63.56 ± 0.3^{c}	33.23 ± 0.5^{b}	0.89 ± 0.4^{b}	2.31 ± 0.2^{a}	3.53 ± 0.6^{b}
HP 1.5 FM 40	63.42 ± 0.4^{d}	33.34 ± 0.5^{a}	0.90 ± 0.1^{b}	2.33 ± 0.4^{a}	3.60 ± 0.6^{a}
HP 0.5 FM 44	63.69 ± 0.2^{b}	33.12 ± 0.4^{c}	0.88 ± 0.6^{b}	2.31 ± 0.5^{a}	3.44 ± 0.1^{c}
HP 1 FM 44	63.54 ± 0.2^{c}	33.25 ± 0.7^{b}	0.90 ± 0.3^{b}	2.31 ± 0.7^{a}	3.51 ± 0.4^{b}
HP 1.5 FM 44	63.41 ± 0.3^{d}	33.35 ± 0.2^{a}	0.90 ± 0.5^{b}	2.32 ± 0.2^{a}	3.62 ± 0.2^{a}

[1]Chemical composition value are expressed as mean ± standard deviation.
[2]Different superscript letter within column are significant different ($p < 0.05$).

The protein content in HP-enriched pasta increased significantly ($p < 0.05$), ranging from 32.78% to 33.35% compared with the control, whereas FMC failed to exhibit a significant effect ($p > 0.05$) on protein content. The enhancement of HP demonstrates the significant protein content of HP (30–40% dry weight) and its incorporation with soy protein isolate and brown rice, synergically improving the overall protein matrix. Microalgal proteins, particularly from HP, demonstrate amino acid profiles similar to high-quality sources like egg and soybean, encompassing all nine essential amino acids (Batista et al. 2013). Additionally, lipid and fiber content of HP could affect protein cross-linking during extrusion. In high-moisture meat analog, HP decreased springiness while increasing hardness, chewiness, and resilience (Huang et al. 2024). These findings demonstrate that protein enrichment was primarily driven by the intrinsic protein contribution of HP, in combination with soy protein isolate, rather than by processing-induced effects such as moisture-related protein denaturation or aggregation. As no significant moisture-related differences were detected, no trend associated with FMC is inferred for protein content.

Increment of HP and FMC (varied between 0.88 to 0.91%) exhibited no significant differences in ash content ($p > 0.05$) but were significantly lower ($p > 0.05$) compared with the control (0.98%). However, the observed reduction in ash content following HP incorporation may be attributed to a dilution effect, whereby the inclusion of HP partially replaces mineral-rich base ingredients, or to altered mineral retention resulting from modifications in the starch–protein matrix during extrusion processing. In agreement with these findings, Molino et al. (2018) indicated that HP could exhibit lower ash content than staple cereals, including barley, rice, wheat, and corn.

Increment of HP and FMC showed no significant effect on fat content ($p > 0.05$), with values ranging from 2.30% to 2.33%, while the control exhibited a significantly lower value ($p < 0.05$) by 2.03%. However, the reduction in fat without HP addition (control) may be attributed to its relatively low lipid content or to matrix effects, such as fat entrapment within protein–fiber networks formed during extrusion, which could reduce lipid extractability. Damiani et al. (2010) reported that HP contains linolelaidic acid (C18:2n6trans) and a generally stable fatty acid profile, with higher light intensity increasing MUFAs and decreasing PUFAs in the neutral lipid fraction.

The increment of HP supplementation resulted in a significant increase in crude fibre content ($p < 0.05$), with values ranging from 3.21% to 3.62%, while lower values were observed in the control group. Accordingly, the increase is due to the fibrous components of HP, specifically cell wall polysaccharides, which enhance total dietary fibre when integrated into the pasta matrix, but no significant effect ($p > 0.05$) was exhibited by FMC treatment. HP exhibits a significant fiber content, predominantly as a polysaccharide–protein complex, comprising approximately 23.6% protein and 64.9% carbohydrate. This composition suggests its potential utility as a natural emulsifier and stabilizer (Yin et al. 2023). Moreover, Dietary fibre from these sources provides health benefits, such as improved glycaemic control, reduced cholesterol levels, enhanced bowel function, detoxification of mutagens, and the promotion of beneficial gut microbiota (Niccolai et al. 2019). This outcome suggests the lack of moisture-related effects further indicates that the extrusion conditions applied did not compromise fiber integrity or induce measurable degradation.

The addition of HP to gluten-free pasta markedly enhanced its nutritional profile, decreasing carbohydrate content and increasing protein and crude fibre levels. Conversely, FMC exhibited no significant effect on the chemical composition for the majority of parameters. The enrichment with HP enhanced macronutrient quality and contributed bioactive compounds, functional proteins, and fibrous polysaccharides, which may offer health benefits. HP supplementation exhibits significant potential

as a functional ingredient for the formulation of nutrient-dense, health-promoting gluten-free pasta products.

Color analysis

The color parameters (CIE L^*, a^*, b^*) of gluten-free pasta were significantly affected ($p < 0.05$) by the supplementation of HP and the level of FMC (Table 3). Lightness (L^*) values varied from 68.68 to 79.49, with the control exhibiting the maximum value of 79.49. Increasing HP concentration consistently decreased L^* values at all moisture levels, attributed to the red–orange carotenoid pigments, mainly astaxanthin, present in HP, which diminished surface reflectance. The minimum L^* value (70.46) was recorded in 1.5% HP at 44% moisture content. Reduced FMC further intensified darkening, likely due to enhanced shear and Maillard browning. Meanwhile, redness (a^*) value significantly increased with the addition of HP, rising from 0.05 in the control to 11.67 in HP 1.5 FM 36, indicating the substantial pigment contribution of astaxanthin. Reduced moisture levels enhanced pigment retention, while increased moisture potentially diluted or partially degraded the pigments. Yellowness (b^*) increased from 12.88 in the control group to 17.11 in HP 1.5 FM 36, indicating the contribution of carotenoids to yellow tones. A minor reduction in b^* at elevated moisture levels indicates reduced pigment stability during hydrated extrusion conditions. The addition of HP enhanced red and yellow hues while reducing lightness, with FMC influencing pigment stability and visual intensity.

Table 3. Color measurement of gluten free pasta[1,2,3].

Sample	L^*	a^*	b^*	Color
Control	79.49 ± 0.4^{a}	0.05 ± 0.3^{j}	12.88 ± 0.2^{j}	
HP 0.5 FM 36	76.83 ± 0.2^{d}	7.18 ± 0.2^{g}	14.29 ± 0.1^{g}	
HP 1 FM 36	72.69 ± 0.5^{g}	9.31 ± 0.1^{d}	15.44 ± 0.5^{d}	
HP 1.5 FM 36	68.68 ± 0.6^{j}	11.67 ± 0.5^{a}	17.11 ± 0.4^{a}	
HP 0.5 FM 40	77.12 ± 0.1^{c}	7.04 ± 0.6^{h}	13.97 ± 0.6^{h}	
HP 1 FM 40	73.27 ± 0.3^{f}	9.14 ± 0.4^{e}	14.53 ± 0.4^{e}	
HP 1.5 FM 40	69.35 ± 0.2^{i}	11.29 ± 0.6^{b}	15.89 ± 0.3^{b}	
HP 0.5 FM 44	78.54 ± 0.5^{b}	6.82 ± 0.5^{i}	13.22 ± 0.5^{i}	
HP 1 FM 44	74.77 ± 0.6^{e}	8.37 ± 0.3^{f}	14.63 ± 0.6^{f}	
HP 1.5 FM 44	70.46 ± 0.7^{h}	10.06 ± 0.5^{c}	15.08 ± 0.4^{c}	

[1]Color value are expressed as mean ± standard deviation.
[2]Different superscript letter within column are significant different ($p < 0.05$).
[3]L*: lightness, a*: redness, b*: yellowness.

The findings are consistent with earlier studies indicating that higher HP content in plant-based meat analogues decreases lightness and results in a darker appearance. Specifically, 1–7% HP enhances beef-like redness, whereas concentrations exceeding 5% lead to excessive darkening, which may adversely affect consumer acceptance (Liu et al. 2023; Huang et al. 2024). Moreover, Hossain et al. (2017) suggested that astaxanthin-rich powders reduced L^* values while increasing a^* and b^* values in cookies, indicating enhanced coloration due to carotenoids. At reduced FMC, elevated extrusion temperatures enhance Maillard reactions, leading to a decrease in L^* and an increase in a^* and b^* due to melanoidin formation (Dalbhagat et al. 2019), while increased moisture levels diminish this effect. HP supplementation exhibited darker, redder, and more yellow hues compared to the control. This variation is attributed to pigmentation, which is influenced by concentration and moisture levels – elements that might affect consumer perception and acceptance of functional, health-oriented pasta products.

The addition of HP markedly affected the colour characteristics of gluten-free pasta, as higher HP concentrations led to decreased lightness (L^*) and increased redness (a*) and yellowness (b*), mainly attributable to the presence of astaxanthin and other carotenoids. FMC further influenced these effects: reduced moisture increased darkening and pigment retention due to enhanced shear and Maillard browning, whereas elevated moisture diminished pigment stability and visual intensity. The findings are consistent with prior research indicating that HP-enriched foods exhibit darker, redder tones, emphasising the significance of pigment concentration and extrusion conditions in influencing the final appearance of the product. The enhancement of red–yellow hues may increase the visual appeal of functional pasta at moderate HP levels; however, excessive darkening could adversely affect consumer acceptance.

Texture profile analysis

The impact of increasing HP concentration and FMC on the texture of gluten-free pasta is illustrated in Fig. 2. Uncooked pasta hardness exhibited a significant increase with elevated HP levels ($p < 0.05$), while a rise in FMC resulted in a decrease in hardness value. The maximum hardness observed in HP treatments was presented by HP 1.5 FM 36 (3.38 kgF), and the minimum was recorded in HP 0.5 FM 44 (1.76 kgF), while all treatments remained below the control (3.80 kgF). The increase in hardness with HP affected by increasing protein and fiber content, which enhances matrix rigidity. In contrast, higher moisture levels facilitate plasticity and reduce shear, resulting in a softer texture. Hossain et al. (2017) revealed that elevating the concentration of astaxanthin-rich powder in wholemeal cookie formulations affected their texture profile, resulting in decreased lightness and changes in hardness. The chewiness of cooked pasta significantly decreased ($p < 0.05$) with increasing high pressure and FMC. The control exhibited the highest value at 10.60 N×mm, whereas HP 1.5 FM 44 recorded the lowest at 1.99 N×mm. Excessively high pressure and increased moisture likely interfere with starch–protein bonding, resulting in a looser structure. This observation is similar to findings that intact high-pressure cells create voids and disrupt fiber alignment, thereby diminishing cohesiveness and elasticity (Liu et al. 2023; Yin et al. 2023). Additionally, higher FMC enhances starch gelatinization and granule swelling, resulting in softer, less dense matrices, while lower moisture leads to firmer, chewier textures (Patria et al. 2020; Seo et al. 2023).

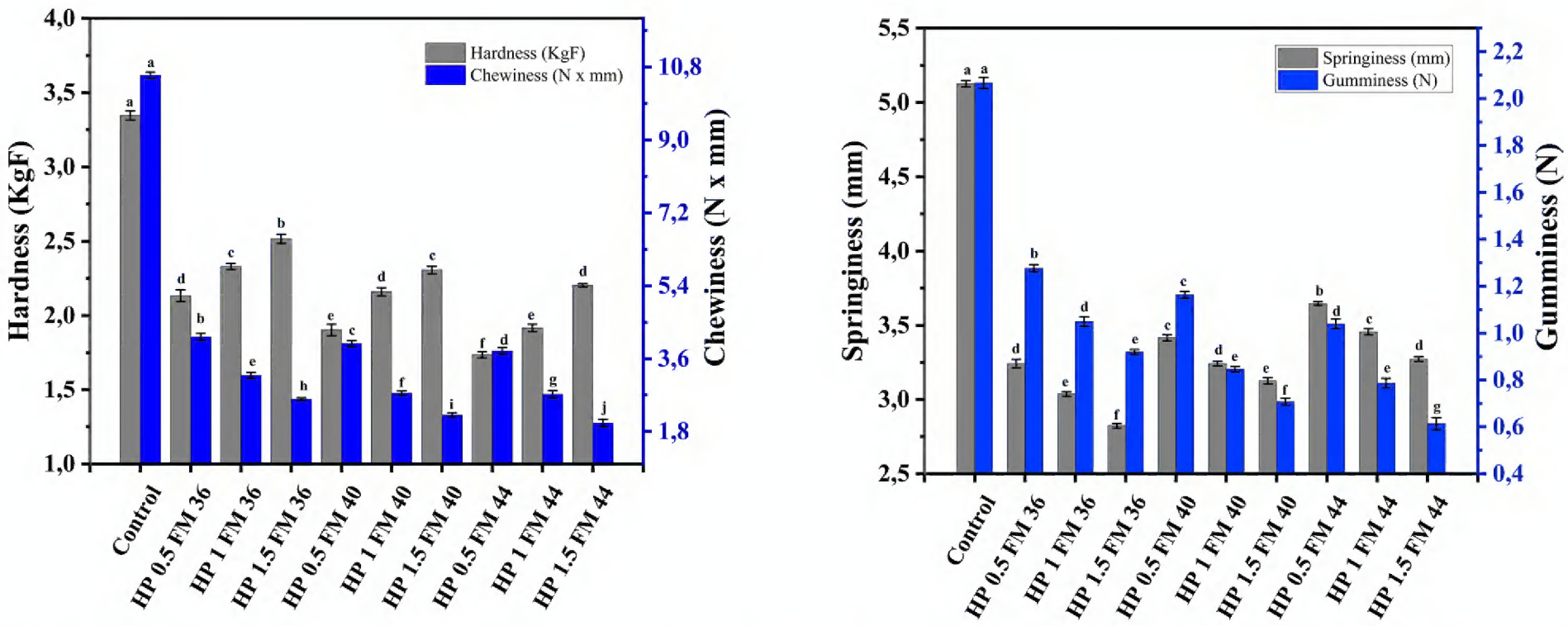

Figure 2. Effect of HP addition and FMC on the textural properties of gluten free pasta. Different letter (a-j) in same color is significant different ($p < 0.05$).

The gumminess of cooked pasta showed significantly decreased ($p < 0.05$) with both increased HP concentration and higher FMC. The control demonstrated the highest value at 2.07 N, and HP 1.5 FM 44 recorded the lowest value at 0.61 N. The gumminess reduction corresponds to HP supplementation, and increased moisture interferes with cohesive gel formation, thus decreasing the energy needed to disintegrate the cooked matrix. Springiness decreased as HP increased, while it increased with higher FMC ($p < 0.05$). The maximum control value recorded was 5.12 mm, whereas the minimum was noted in HP 1.5 FM 36 at 2.84 mm. The diminished springiness observed with HP can be attributed to its fibrous content, which disrupts the formation of a resilient gel network. In contrast, increased moisture levels facilitate starch gelatinization and protein unfolding, thereby enhancing structural recovery during deformation. The findings correspond with reports indicating that astaxanthin-rich HP modifies rheological properties (Liu et al. 2023) and that increased extrusion moisture diminishes hardness, gumminess, and chewiness in plant protein-based analogues (Kantanen et al. 2022).

Elevated concentrations of HP increased the hardness of uncooked gluten-free pasta, attributed to greater protein and fibre content, whereas increased FMC decreased hardness by enhancing plasticity. In cooked pasta, increased HP levels resulted in a decline in chewiness, gumminess, and springiness, while higher moisture content softened the texture by enhancing starch gelatinisation and decreasing cohesiveness. HP incorporation enhanced structural rigidity prior to cooking but diminished elastic recovery and cohesiveness post-cooking, with FMC serving as a crucial modulator of texture.

Water Absorption Index (WAI) and Water-Soluble Index (WSI)

The effects of HP concentration and FMC on the water absorption index (WAI) and water-soluble index (WSI) of gluten-free pasta are shown in Fig. 3. The WAI showed the minimum value observed in the control group (2.93 g/g) and the maximum in HP 1.5 FM 36 (3.62 g/g). HP supplementation resulted in a slight increase in WAI. Statistical analysis revealed that HP incorporation at 0.5%, 1.0%, and 1.5% significantly increased WAI compared to the control ($p < 0.05$). However, no statistically significant differences in WAI were observed among the HP-supplemented samples themselves, regardless of HP concentration or FMC ($p > 0.05$). This indicates that while the presence of HP contributes to enhanced water absorption relative to the control formulation, further increases in HP concentration do not produce proportional or statistically measurable changes in WAI. Similarly, variations in FMC (FM36, FM40, and FM44) did not result in significant differences in WAI within the same HP level ($p > 0.05$). This suggests that, within the investigated moisture range, FMC did not exert a dominant influence on the water-holding capacity of the pasta matrix. The absence of moisture-related ef-

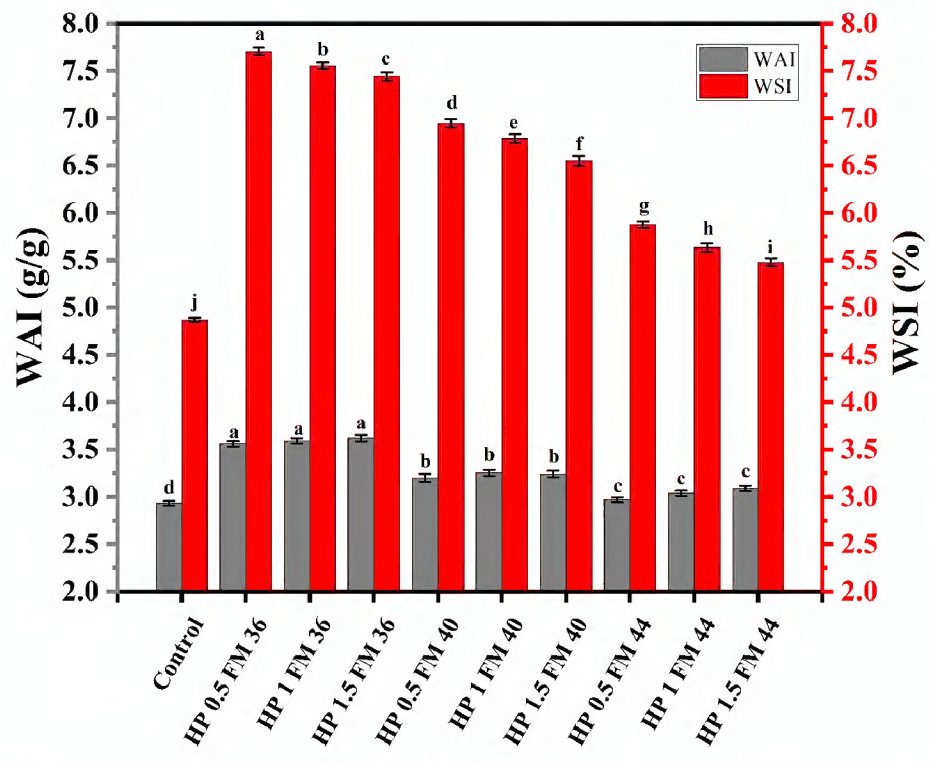

Figure 3. Water absorption index (WAI) and water-soluble index (WSI) of gluten free pasta. Different letter (a-j) in same color is significant different ($p < 0.05$).

fects may be attributed to the relatively narrow moisture interval applied, which was sufficient to ensure adequate starch gelatinization but insufficient to induce substantial differences in macromolecular restructuring affecting water uptake. Comparable trends have been observed in microalgae-enriched extrudates, indicating that WAI remained unchanged with the addition of algae, yet diminished with increased moisture content (Nagaraju et al. 2020; Alcaraz et al. 2021).

The WSI varied between 4.87% and 7.71%, with the control group exhibiting the minimum value of 4.87%. All samples supplemented with HP demonstrated significantly reduced WSI values ($p < 0.05$), with a progressive decline noted as HP concentration increased. Reducing of WSI indicates that the fibrous and protein-rich components of HP may withstand thermal and mechanical degradation during extrusion, thereby restricting the release of soluble fractions. The WSI experienced a slight decline with increased FMC, likely attributable to decreased shear and thermal intensity, which reduces macromolecular fragmentation. Accordingly, decreases in WSI with higher microalgae incorporation or FMC have been documented for feed pellets (Alcaraz et al. 2021), rice–mung bean snacks (Sharma et al. 2017), and sorghum-based extrudates (Tadesse et al. 2019).

HP supplementation resulted in a slight increase in the water absorption index (WAI); however, this effect was not statistically significant. In contrast, elevated FMC led to a reduction in WAI, attributed to decreased shear and starch gelatinisation. The water-soluble index (WSI) exhibited a significant decrease with rising HP concentration, indicating the stability of its protein- and fiber-rich matrix during the extrusion process. Increased FMC resulted in a reduction of WSI due to the restriction of macromolecular degradation. HP improved water retention and decreased solubility, with FMC serving as a significant factor affecting both measures.

Cooking quality measurement

The cooking time of gluten-free pasta varied between 6.0 and 10.5 minutes. The control sample exhibited the longest OCT at 10.5 min, which was significantly greater than that of all HP-supplemented samples ($p < 0.05$). The data indicated a slight increase in OCT with higher HP levels, but the effect was not statistically significant ($p > 0.05$). The maximum OCT for HP treatments was recorded at 9.0 min in HP 1.5 FM 36, whereas the minimum was 6.0 min in HP 1.5 FM 44. The prolonged OCT at elevated HP levels might indicate the fibrous, protein-dense characteristics of HP, which could obstruct water absorption and postpone starch gelatinization during cooking. In contrast, increasing FMC markedly decreased OCT ($p < 0.05$), presumably as a result of improved starch gelatinization and matrix softening during the extrusion process. Similarly, OCT value were affected by the addition of high-fiber algae to pasta (Ribeiro et al. 2022) and with elevated FMC during extrusion cooking (Zambrano et al. 2022).

WAR values were affected by increament of HP and varied between 137.5% and 195.8%, with the control group showing the highest value (195.8 ± 0.3%) and HP 1.5 FM 44 the lowest (137.5%). This difference was statistically significant ($p < 0.05$). HP supplementation consistently decreased WAR across all moisture levels, likely attributable to its high dietary fiber and protein content, which binds water within the matrix and restricts penetration into starch granules during cooking. FMC significantly influenced WAR ($p < 0.05$), with increased moisture levels correlating with reduced WAR. This relationship indicates diminished mechanical shear and starch damage during extrusion, leading to lower water absorption during cooking. This trend indicates the significant water-binding capacity of dietary fiber in HP (Williams et al. 2019; He et al. 2022) and reduced starch damage at elevated FMC, as reduced mechanical shear restricts water absorption during cooking (Gandhi et al. 2018).

Cooking loss (CL) of the extrudates ranged from 5.13% to 8.79%, with the control exhibiting the lowest value (5.13%) and the sample with 1.5% HP at 44% FMC showing the highest (8.79%). While HP supplementation tended to increase CL, this effect was not statistically significant ($p > 0.05$), whereas higher FMC significantly increased CL ($p < 0.05$). The observed increase in CL with higher FMC can be attributed to weakened structural integrity of the extrudate matrix. Higher moisture reduces the extent of protein denaturation and starch gelatinization during extrusion, leading to a less cohesive starch–protein network (Bresciani et al. 2022; Zhang et al. 2025). Incorporation of microalgae (HP) particles may further disrupt matrix continuity, as their fibrous, pigment-rich, and rigid cell walls hinder proper integration with the starch–protein network, thereby reducing compactness and facilitating the leaching of water and soluble components during cooking (Ungureanu-Iuga and Mironeasa 2023). These findings indicate that both microalgae addition and FMC play critical roles in modulating the structural integrity and water retention of the extrudates.

HP supplementation decreased cooking time and water absorption ratio, while marginally increasing cooking loss, indicating its elevated fibre and protein content,

Table 4. Cooking quality of gluten free pasta[1,2,3].

Sample	OCT (min)	WAR (%)	CL (%)
Control	10.5 ± 0.2^{a}	195.8 ± 0.5^{a}	5.13 ± 0.3^{d}
HP 0.5 FM 36	8.5 ± 0.5^{c}	195.3 ± 0.4^{a}	8.22 ± 0.3^{c}
HP 1 FM 36	8.5 ± 0.4^{c}	191.6 ± 0.5^{b}	8.25 ± 0.2^{c}
HP 1.5 FM 36	9 ± 0.1^{bc}	188.2 ± 0.3^{c}	8.29 ± 0.5^{c}
HP 0.5 FM 40	7 ± 0.5^{e}	176.4 ± 0.2^{d}	8.48 ± 0.4^{b}
HP 1 FM 40	7 ± 0.7^{e}	170.8 ± 0.3^{e}	8.51 ± 0.1^{b}
HP 1.5 FM 40	7.5 ± 0.5^{de}	166.6 ± 0.1^{f}	8.54 ± 0.3^{b}
HP 0.5 FM 44	6 ± 0.4^{f}	149.3 ± 0.2^{g}	8.73 ± 0.2^{a}
HP 1 FM 44	6 ± 0.3^{f}	142.1 ± 0.6^{h}	8.76 ± 0.5^{a}
HP 1.5 FM 44	6 ± 0.3^{f}	137.5 ± 0.4^{i}	8.79 ± 0.2^{a}

[1]Cooking quality value are expressed as mean ± standard deviation.
[2]Different superscript letter within column are significant different ($p < 0.05$).
[3]OCT: optimum cooking time, WAR: water absorption ratio, CL: cooking loss.

which modifies starch–protein interactions. Increased FMC reduced cooking time and WAR, while significantly elevating cooking loss due to a less compact matrix. HP and FMC significantly affected pasta cooking behaviour by altering water binding, starch gelatinisation, and matrix stability.

Microstructure analysis

Microstructural analysis of cooked gluten-free pasta (Fig. 4B) indicated that both HP supplementation and FMC significantly affected the integrity of the starch–protein network. Control samples displayed a dense, continuous matrix with few voids, suggesting robust starch–protein interactions commonly associated with gluten. Conversely, elevated HP levels led to more heterogeneous and porous structures, likely because the fibrous, pigment-rich HP particles function as inert fillers that compromise network compactness and interfacial bonding.

At 36% FMC, the microstructure exhibited significant fragmentation and voids, indicating increased shear and thermal stress during the extrusion process. These conditions facilitated starch gelatinization but also weakened network cohesion, particularly with HP present. Increased moisture levels (40–44%) resulted in smoother, less fractured matrices, due to diminished shear stress and improved dough plasticity. High HP inclusion (1.5%) consistently resulted in network discontinuities, indicating that HP inherently restricts matrix formation, regardless of moisture content. This observation is consistent with previous study reported by McPherson and Jane (2000) and Mitrus et al. (2017), indicating that reduced FMC during extrusion enhances fragmentation and facilitates a more open, cellular structure via improved gelatinization.

The observed microstructural modifications have direct implications for functional and nutritional properties. The less compact, more permeable matrices in HP-enriched samples likely contributed to increased cooking loss, decreased hardness in uncooked pasta, and reduced gumminess in cooked samples. Moderate HP addition slightly improved springiness, potentially due to the protein fraction of HP; however, this effect was reduced at higher moisture levels due to diminished network cohesion. Beyond textural effects, the disrupted starch–protein network may also influence digestibility. The physical interference of HP particles can limit enzymatic access to starch granules, slowing carbohydrate hydrolysis and potentially reducing the glycemic index of the pasta. Thus, HP supplementation not only enhances antioxidant activity but may also provide functional benefits related to blood glucose management.

HP supplementation altered the starch–protein network of gluten-free pasta, resulting in more porous and heterogeneous matrices in contrast to the dense structure observed in the control. Decreased FMC enhanced fragmentation due to elevated shear, whereas increased moisture enhanced plasticity and diminished fractures. The less compact networks in HP-enriched samples account for increased cooking loss, diminished hardness, and reduced gumminess, whereas moderate HP addition resulted in a slight enhancement of springiness. Both HP and FMC significantly influenced microstructure, directly affecting the functional and textural properties of the pasta.

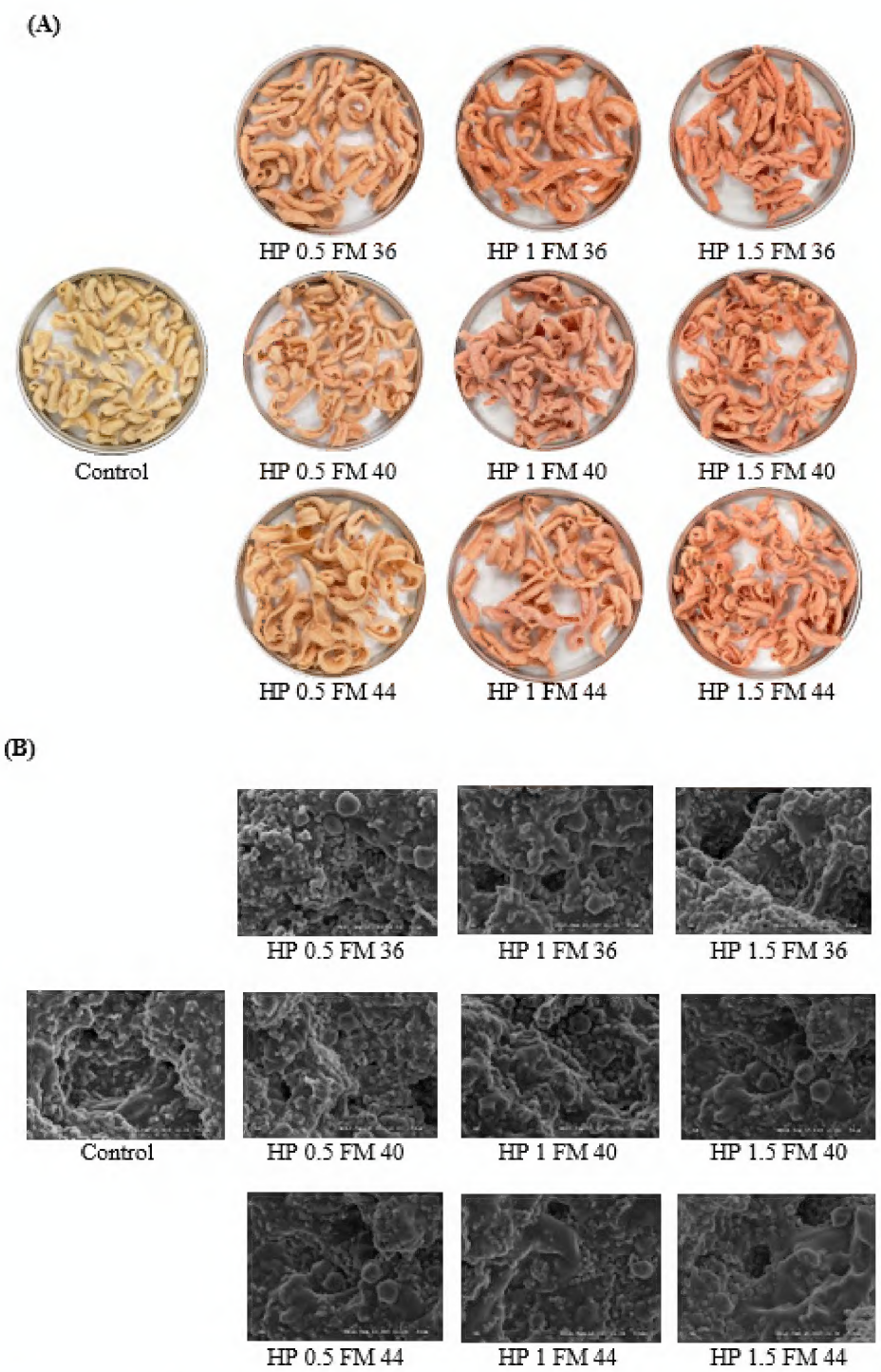

Figure 4. (**A**) dry gluten free pasta sample surface images (**B**) microstructure of cooked pasta.

DPPH free radical scavenging assay

DPPH free radical scavenging activity of both raw and cooked gluten-free pasta (Fig. 5) showed a significant enhancement with HP supplementation ($p < 0.05$). In raw pasta, scavenging activity ranged from 41.6% (control) to 64.1% (HP 1.5 FM 44%), while in cooked pasta it decreased to 28.2% (control) and 40.7% (HP 1.5 FM 44%). The increase in antioxidant activity is attributed to the high astaxanthin and carotenoid content in HP, which are effective free radical scavengers.

The observed reduction in DPPH activity after cooking is primarily due to the thermal sensitivity of astaxanthin. During the heating or boiling phase, such as during pasta cooking, astaxanthin can undergo thermal degradation due to exposure to high temperatures and an aqueous environment. This degradation involves oxidation of the

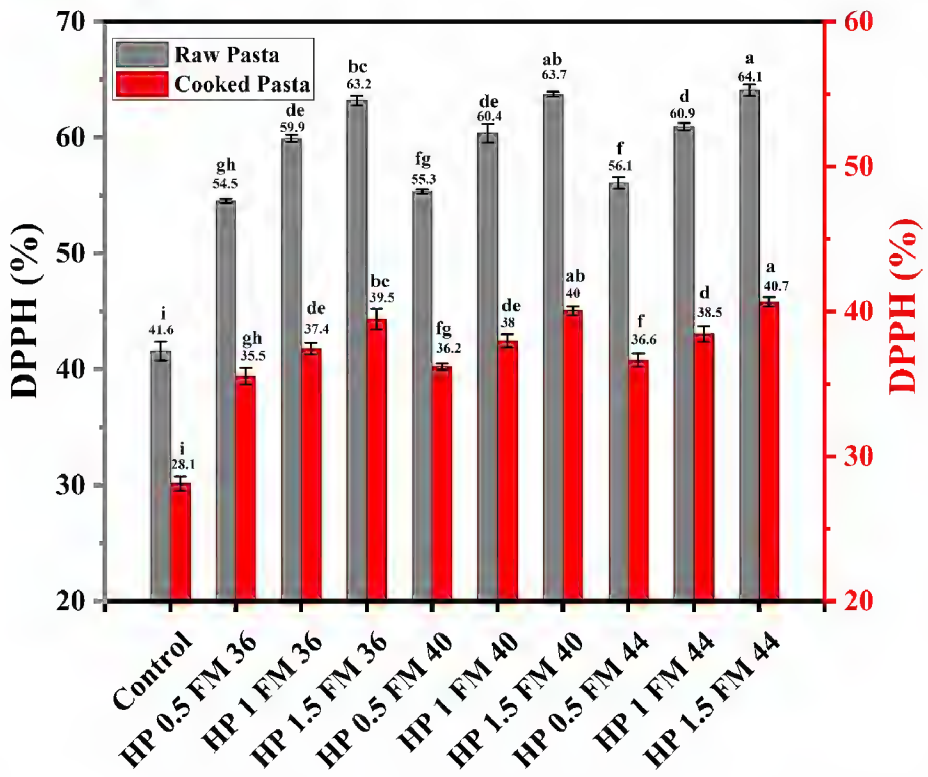

Figure 5. DPPH free radical scavenging results of raw pasta and cooked pasta. Different letter (a-i) in same color is significant different ($p < 0.05$).

pigment molecules and disruption of the conjugated double bonds that are critical for its free radical-scavenging activity, resulting in a reduction of antioxidant capacity (Zhang et al. 2022). Despite this thermal loss, HP supplementation still significantly improved antioxidant activity in cooked pasta compared to the control, indicating that even after cooking, HP can enhance functional properties. FMC variations (36–44%) did not significantly affect DPPH activity ($p > 0.05$), and no significant HP × FM interaction was observed, suggesting that the antioxidant effect of HP is robust across the tested moisture levels. These findings emphasize the importance of considering astaxanthin thermal stability when making functional claims for cooked pasta products.

Cooking resulted in a notable decrease ($p < 0.05$) in DPPH scavenging activity relative to raw pasta, aligning with the thermal degradation and leaching of carotenoids, including astaxanthin. The findings are supported by previous studies (Oslan et al. 2021; Rizzardi et al. 2022; Habtegebriel and Valdramidis 2025), indicating that minimal HP concentrations could significantly enhance the antioxidant profile of food products. The increased antioxidant capacity is mainly due to astaxanthin's role as an electron and hydrogen donor and its synergistic interaction with other polyphenols in HP. The increment HP supplementation (0.5–1.5%) hypothetically enhances the antioxidant properties of gluten-free pasta without necessitating changes in FMC. However, the cooking process notably affects the retention of antioxidant compounds.

HP supplementation markedly improved the DPPH free radical scavenging activity in both raw and cooked gluten-free pasta, thereby affirming the substantial antioxidant role of astaxanthin and carotenoids. The FMC exhibited no influence, suggesting that enhancements in antioxidants were exclusively attributed to HP. Cooking resulted in decreased activity relative to raw samples due to carotenoid degradation and leaching; however, HP-enriched pasta consistently exhibited higher antioxidant capacity compared to the control. Minimal inclusion of HP significantly enhanced the functional health value of gluten-free pasta.

Sensory evaluation

Overall, the increment of *HP* and FMC significantly influenced the sensory properties of gluten-free pasta (Fig. 6). The HP supplementation (0.5–1.0%) at 40% FMC resulted in significant enhancements in taste, color, and aroma ($p < 0.05$), achieving peak scores of 4.4, 4.1, and 4.2, respectively, but control values depicted low scores of 3.5, 2.9, and 2.9. The improvements are linked to the astaxanthin-rich pigments in HP, which enhance visual appeal while maintaining flavor integrity (Villaró et al. 2021). In contrast, increased HP inclusion (1.5%) or higher FMC (44%) resulted in lower sensory scores, especially regarding color and aroma, presumably due to excessive pigmentation and heightened marine/vegetal characteristics. Accordingly, Baune et al. (2025) suggested that microalgae concentrations exceeding 2% reduce consumer acceptance because of pronounced algal or fishy flavors.

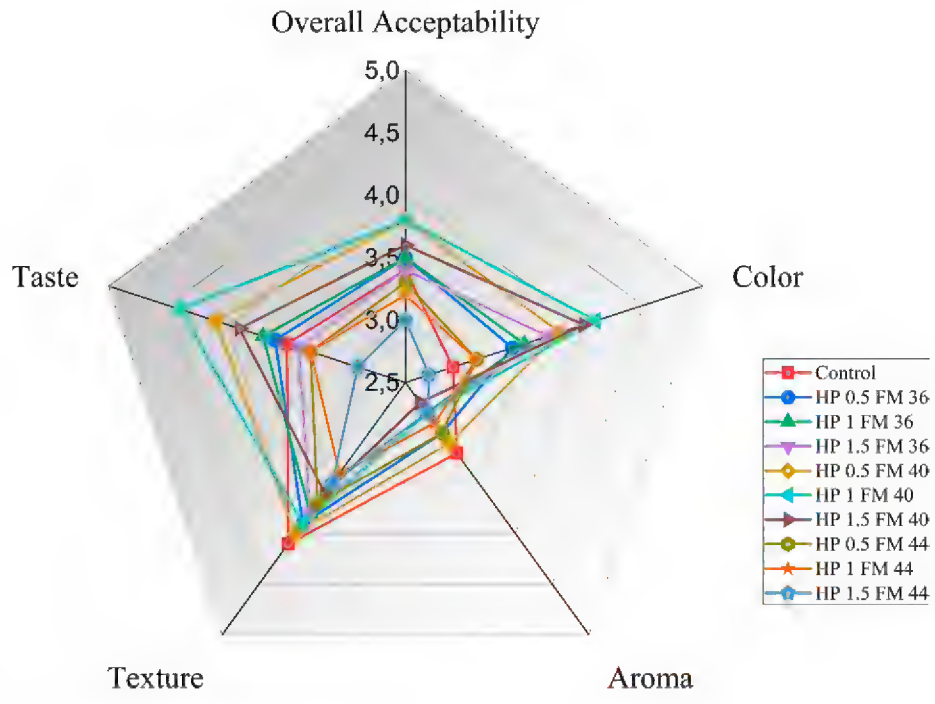

Figure 6. Sensory evaluation result of gluten free pasta.

Texture scores were highest in the control group (4.1) and decreased with the addition of HP; however, increased FMC partially alleviated this effect. Microalgae-enriched pasta exhibited greater flavor intensity and unique sensory profiles than commercial alternatives. Overall acceptability reached a maximum of 3.8 for HP 0.5 FM 40 and HP 1 FM 40, suggesting that moderate HP supplementation improves consumer-perceived quality while maintaining texture integrity when paired with optimal FMC.

Moderate HP supplementation (0.5–1.0%) at 40% FMC significantly enhanced the sensory quality of gluten-free pasta, improving taste, colour, and aroma relative to the control group. Higher HP levels (1.5%) and increased moisture (44%) resulted in decreased scores attributed to excessive pigmentation and heightened marine-like notes. The control exhibited optimal texture preservation, while balanced moisture levels allowed for maintenance at ac-

ceptable standards. Optimal incorporation of HP at moderate levels improved visual appeal and flavour, thereby facilitating consumer acceptance.

Pearson correlation matrix

The Pearson correlation matrix (Fig. 7) demonstrated strong positive correlations between DPPH radical scavenging activity and the contents of protein, fiber, and fat ($r \geq 0.88$). This suggests that HP-enriched samples with elevated levels of these bioactive compounds displayed improved antioxidant capacity. The DPPH exhibited a strong correlation with a^* ($r = 0.97$), indicating that heightened red pigmentation, resulting from HP-derived astaxanthin, is associated with enhanced antioxidant potential. A significant negative correlation was observed between DPPH and lightness (L^*) ($r = -0.66$), indicating that darker, more pigmented pasta exhibits enhanced antioxidant properties.

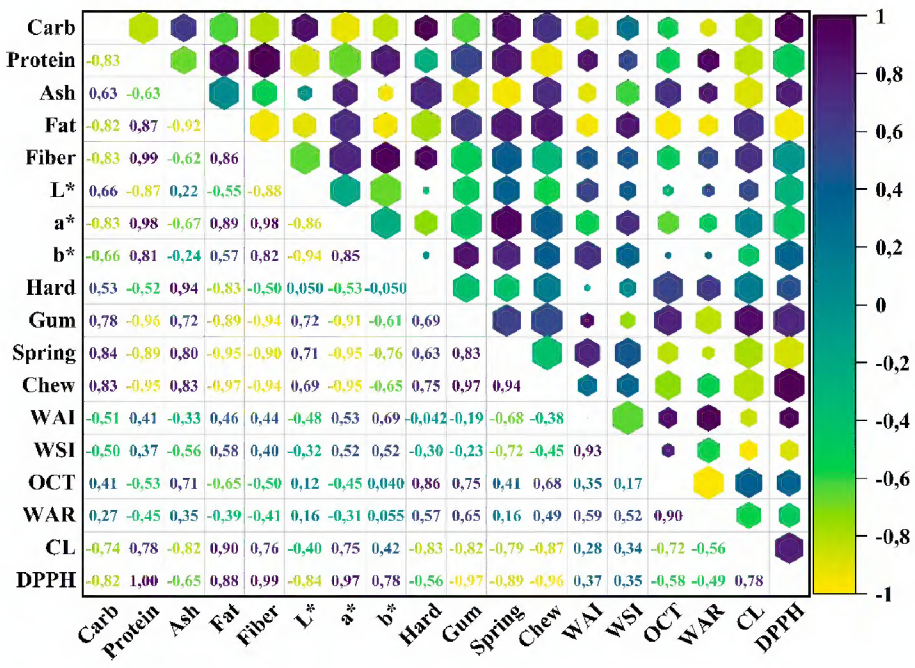

Figure 7. Result of Pearson correlation matrix responses.

The cooking quality parameters were correlated with the proximate composition. The optimal cooking time (OCT) and cooking loss (CL) exhibited a positive correlation with protein and fiber contents ($r = 0.71–0.78$), suggesting that a denser, fiber-rich matrix necessitates extended cooking durations and leads to increased solids loss. The water absorption ratio (WAR) negatively correlated with protein and fiber ($r = -0.48$ to -0.53), indicating a diminished swelling capacity in these reinforced matrices. Texture attributes – hardness, gumminess, and chewiness – exhibited significant positive correlations with protein and fiber ($r = 0.83–0.94$), indicating that increased structural biopolymers from HP fortification enhanced the pasta matrix.

Conclusion

HP supplementation (0.5–1.5%) significantly boosted protein (32.78–33.35%), fiber (3.21–3.62%), and antioxidant activity (DPPH up to 64.1% raw, 40.7% cooked), while lowering carbohydrates compared to wheat protein control. Optimal conditions – HP 0.5–1.0% at 40% feed moisture – yielded the highest sensory acceptability (overall 3.8/5), enhanced color (redder/yellower tones), and suitable texture/hardness for storage stability. Cooking quality improved with shorter times (6–9 min vs. 10.5 min control), though higher moisture increased loss; microstructure showed porous networks explaining functional traits. These results demonstrate HP as a viable, sustainable functional ingredient for nutrient-dense gluten-free pasta, addressing celiac needs and health trends with astaxanthin-driven benefits like better glycaemic control and radical scavenging. Cold extrusion preserves bioactive, enabling scalable production of visually appealing, acceptable products outperforming basic gluten-free bases.

Limitations

This study has several limitations that should be acknowledged. The sensory evaluation was conducted using a relatively small panel of untrained young adults, which limits the generalizability of consumer acceptance results to the broader gluten-free population. Additionally, the antioxidant capacity was evaluated using in vitro assays, which do not fully represent in vivo bioavailability or physiological effects. The potential impact of HP incorporation on starch digestibility and glycemic response was inferred from microstructural observations rather than directly measured. Future studies should include in vitro digestion models, glycemic index assessment, and larger, more diverse consumer panels, including individuals with diagnosed gluten intolerance or celiac disease, to validate the nutritional and functional claims at a commercial scale.

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Conflicts of Interest

The authors declare no conflict of interest.

Author contributions

Domas Galih Patria: Conceptualization, Methodology, Writing – original draft, review, & editing. Muhammad Dzaky Irsyad Naufal: Data curation, Investigation, Methodology, Resources. Cindytia Selvina Bernas: Data curation, Investigation. Annur Ahadi Abdillah: Methodology, Writing – original draft, review, & editing. Jenshinn Lin: Conceptualization, Funding, Supervision.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
No AI tools were used in the preparation of this manuscript.

Data collection and analysis

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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